Title Update on feline hemoplasmosis

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Key Words (5–8) Mycoplasma haemofelis; clearance; haemoplasma; fluoroquinolone; doxycycline

Disclosure statement Employed by Langford Vets, a wholly owned subsidiary of University of Bristol, whom offer a quantitative PCR assay for the detection of canine and feline haemoplasmas. Author's PhD on the molecular characterization of companion animal hemoplasmas was part funded by Zoetis (previously Pfizer Animal Health).

Abstract/Summary [this will be used for indexing services and does not appear with article]
The wall-less, hemotropic, mycoplasma species *Mycoplasma haemofelis,* '*Candidatus* Mycoplasma turicensis' and, to a lesser extent, '*Candidatus* Mycoplasma haemominutum' have the potential to induce clinical hemolytic anemia in infected cats. Prevalence varies markedly between infecting species, complicated by a chronic carrier state. Accurate and prompt confirmation of infection and identification of the infecting hemoplasma species enables appropriate antibiotics (e.g. tetracycline; fluoroquinolone) to be prescribed. Although cats with hemoplasmosis respond rapidly to antibiosis and supportive care, initial monotherapy treatment rarely results in clearance of infection. A protocol now exists for the clearance of the most pathogenic feline hemoplasma *M. haemofelis*.

Key Points (3–5)

- Hemoplasmosis due to *Mycoplasma haemofelis* infection is an uncommon cause of potentially fatal, moderate-severe hemolytic anemia in cats.
- '*Candidatus* Mycoplasma haemominutum' infection, detected in approximately 20% of cats, can result in mild anemia; however, a subclinical carrier state is common.
- Decision to treat should be based on accurate diagnosis of hemoplasma infection, including speciation, using molecular diagnostic techniques (i.e. PCR).
- Tetracyclines (e.g. doxycycline) and fluoroquinolones (e.g. marbofloxacin; pradofloxacin) are considered appropriate choices of antibiotics for hemoplasmosis
- Monotherapy rarely results in persistent clearance of infection; however, the chronic carrier state is uncommonly of clinical concern

Introduction

Hemotropic mycoplasmas (hemoplasmas) are wall-less bacteria that parasitize red blood cells. They can induce potentially life-threatening hemolytic anemia in a wide variety of mammals, including the domestic cat, where they are the agent of feline infectious anemia.

What is in a name?

Hemoplasmas were first described in laboratory rodents in the 1920s, appearing in association with anemia following splenectomy [1-3]. Originally thought to be members of the *Bartonella* genus due to

their hemotropic properties, small size (<1µm), and suspected role of arthropods in their transmission, their re-classification within the order Rickettsiales in the family *Anaplasmataceae* was suggested a decade later due to their uncultivatable status and lack of cutaneous lesions [4]. However, questions still remained as to the correct positioning of these organisms within the family *Anaplasmataceae* due to their lack of a cell wall, lack of flagellae, inability to invade erythrocytes, and apparent antibiotic sensitivities (resistant to penicillin; sensitive to tetracyclines). Following the advent of DNA sequencing and phylogenetic analysis based upon ribosome gene sequence comparisons, many of the hemoplasmas became officially reclassified as mycoplasmas [5-7]. Reclassification remains incomplete, as the first description of the rodent hemoplasma *Eperythrozoon coccoides* pre-dates that of the type species for the *Mycoplasma* genus *Mycoplasma mycoides* [8].

In 1942 the first description of such bacteria in association with anemia in a cat appeared, with the name *Eperythrozoon felis* [9]. Further descriptions, using the name *Haemobartonella felis*, appeared shortly afterwards [10-12]. Outside the USA, this feline hemoplasma continued to be referred to as *E. felis* into the 1970's before it became universally known as *H. felis*, with subsequent re-classification as *Mycoplasma haemofelis* at the turn of the 21st century [7,13]. Of note, by contrast with rodents and dogs, clinical disease was reported in cats that had not been splenectomized.

Prior to gene-based phylogenetic analysis, it had been recognized that '*H. felis*' existed in different forms of differing pathogenicities, with the "large" form (a.k.a. Illinois, Florida, Ohio and Oklahoma strains) most frequently associated with disease compared to the "small" form (a.k.a. California and Birmingham strains) [14]. Ribosomal gene analysis (16S rRNA gene and *rnpB*) confirmed the presence of two distinct organisms, *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' [7,13,14]. More recently a further feline hemoplasma '*Candidatus* Mycoplasma turicensis' was described in association with hemolytic anemia [15]. All three of these organisms have since been described worldwide, sometimes in combination, with variable prevalences [16]. These prevalence studies, mostly based on convenience samples, have also described the detection of '*Candidatus* Mycoplasma haematoparvum' and '*Candidatus* Mycoplasma haematoparvum'-like organisms in cats [17,18]; however, their role in disease is unknown.

Clinical relevance

Many of the clinical signs reported with hemoplasmosis (lethargy, weakness, depression, collapse, pallor, tachycardia, dyspnea/tachypnea, cardiac 'hemic' murmur, hepatosplenomegaly, lymphadenopathy, dehydration, fever, weight loss, pica, icterus) result from anemia or the underlying immune-mediated process, which can be fatal in severe cases.

The presence of a chronic carrier state has limited the ability of epidemiological studies to associate hemoplasma infection with disease. In experimental feline studies, acute infection with *M. haemofelis* often results in severe hemolytic anemia in previously immunocompetent cats [19-21]. However, at other times, only mild anemia is seen and it has been suggested that age may play a role in outcome, with younger cats more likely to develop more severe anemia. Although experimental infection with '*Ca*. M. haemominutum' and '*Ca*. M. turicensis' can result in a decreased hematocrit, they are infrequently associated with clinical signs in the absence of concurrent disease or immunosuppression. It should be noted that these studies comprise only handful of hemoplasma isolates, and that it is unclear whether strains of differing pathogenicity exist within an individual species. Experimental studies have also

demonstrated protective immunity against re-challenge with the same hemoplasma species for both *M. haemofelis and* '*Ca.* M. turicensis' [22,23].

Consistent with an immune-mediated hemolytic anemia, auto-agglutination or positive Coombs' testing may be detectable during acute *M. haemofelis* infection [21]. However, hemoplasmosis remains an infrequent cause of immune-mediated hemolytic anemia, positive auto-agglutination results or a positive Coombs' test [24].

Diagnosis

Blood should be collected for analysis prior to the administration of any treatments, particularly antibiotics with known activity against mycoplasmas (i.e. tetracyclines and fluoroquinolones).

Although, cytological detection of organisms during acute hemoplasmosis could be considered as a point-of-care test, this is limited by the experience of the slide reviewer, as misdiagnosis can potentially arise from artefact (stain precipitate, poor slide preparation), Howell-Jolly bodies and other infecting species. One study demonstrated that cytology has a sensitivity of 11.1 %, with a specificity of 84 % [25] while another reported a detection rate of 37.5 % based on blood smear examination, compared to 100 % for PCR in cats experimentally infected with *M. haemofelis* and *'Ca.* M. haemominutum' [26]. In addition, the presence of the anti-coagulants EDTA and heparin have been suggested to result in the disassociation of hemoplasmas from erythrocytes over time, necessitating the preparation of fresh blood smears if assessment for their presence is desired [27,28]. Cytological visualization of organisms may not be possible for some hemoplasma species; for example, in cats experimentally infected with '*Ca.* M. turicensis' blood copy numbers remain very low, even during acute infection [15,29].

Accurate diagnosis is currently reliant on the detection of bacterial DNA using PCR assays, which have been repeatedly demonstrated to be both more sensitive and specific than cytology [14,19-21,26,30]. Newer quantitative PCRs determine hemoplasma numbers within samples collected [18,31]. In common with most PCRs, due to small volumes of reagents utilized in these assays, the limit of detection for a typical assay is 200 organisms per mL blood. This is based on the detection of a single copy of a target gene within the 5µL purified DNA added to the PCR reaction, where DNA is purified from blood and eluted in an equivalent volume, and where the gene is present in a single copy within the organism.

In a US study of cats with signs consistent with hemoplasmosis 4.8% were found to be infected with *M. haemofelis*, 23.2% to be infected with '*Ca.* M. haemominutum' and 6.5% to be infected with '*Ca.* M. turicensis', of which 6.5% represented co-infections [32]. A similar UK study found lower prevalences of hemoplasma infection, with 2.8% infected with *M. haemofelis*, 11.2% infected with '*Ca.* M. haemominutum' and 1.7% infected with '*Ca.* M. turicensis', of which 1.6% represented co-infections [31]. A higher prevalence of *M. haemofelis* (7.6%) was reported in a convenience sample population of cats, where 21.7% were also infected with '*Ca.* M. haemominutum', although hematological parameters were not determined for these cats [33]. However, where hemoplasma prevalence has been compared between anemic and non-anemic cats, some authors have found no detectable difference [24,34], while another study reported that '*Ca.* M. haemominutum'-infected cats were less likely to be anemic [18].

Due to the presence of a chronic carrier state, detection of lower pathogenicity hemoplasmas '*Ca.* M. haemominutum' and '*Ca.* M. turicensis' in an anemic cat should prompt continued investigation of concurrent contributory factors to the etiopathogenesis of the anemia. However, knowledge of their presence is of use when considering treatment options, particularly if these would involve immune suppression e.g. in the treatment of idiopathic immune-mediated hemolytic anemia or lymphoma, or where no additional factors are identified.

It should be noted that the design of highly species-specific PCR assays can limit the detection of novel or incompletely described hemoplasmas. In the rare event that clinical suspicion persists, further advice from the laboratory performing the assay should be sought.

Treatment

When to treat

Cats with severe anemia resulting in cardiovascular compromise, including those whose anemia is caused by hemoplasmosis, are fragile and require careful management pending confirmation of underlying etiology. However, confirmation of diagnosis by PCR is often subject to a time delay, as samples are submitted to external commercial laboratories. Clinical hemoplasmosis, when confirmed, can often be successfully managed with a course of an appropriate antibiotic (see below), in conjunction with basic supportive care [19,35-37].

Despite clinical resolution of hemoplasmosis, infection will only be eliminated in a minority of cases, and some cats can spontaneously clear the infection without antibiotic treatment. It should be noted that the majority of cats found to be infected with hemoplasmas in prevalence studies, particularly those infected with '*Ca.* M. haemominutum' or '*Ca.* M. turicensis', were likely asymptomatic chronic carriers and that, for *M. haemofelis* and '*Ca.* M. turicensis' at least, re-challenge with the original infecting species does not appear to result in clinical disease [22,23].

In some situations, clearance of infection may be desirable in addition to clinical cure. For example, when the cat is immunocompromised by concurrent infection (particularly by retroviruses feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV)), as a result of treatments administered such as chemotherapy, or following splenectomy. Clearance of infection may also be considered where the cat represents a risk to either other cats by both horizontal or vertical transmission, or to an immunocompromised owner [38]. Although horizontal spread remains the most likely route of infection, a possible case of vertical spread from queen to litter of kittens resulting in clinical anemia has been described [39]; unfortunately this study pre-dated molecular confirmation of infection and hemoplasma speciation. Molecular techniques have more recently strongly supported vertical transmission in ruminant species [40,41]. Zoonotic *M. haemofelis* infection has been described in an HIV-positive man with concurrent *Bartonella henselae* infection [38]. The source of infection was suspected to have been one of the *M. haemofelis*-infected cats in the household. Human infections with swine and ovine hemoplasmas have also been reported [42,43]. Finally, as was the case in the experimentally hemoplasma-infected cats included in a recent study [44], clearance of infection may be a requirement of re-homing.

The consequences of the chronic hemoplasma infection ('carrier state') are poorly understood. Anemic-stress has been shown to be a trigger for myelogenous leukemia in susceptible rodents (e.g. irradiated rats and leukemia-prone RF strain of mice) [45,46]. One early experimental study suggested

that cats infected simultaneously with FeLV and *M. haemofelis* were more likely to develop FeLV viremia and aplastic anemia than those not infected with *M. haemofelis* [47]. In another, pre-existing retrovirus infection (FeLV or FeLV/FIV) in apparently healthy cats potentiated the severity of anemia following *'Ca.* M. haemominutum' infection [48]. The latter study also reported a high incidence of myeloproliferative disease (e.g. leukemia, myelodysplastic syndrome, lymphoma); however, the absence of a control population of hemoplasma uninfected cats limited conclusions. Further studies are required to determine the long-term impact of chronic hemoplasma infection on the risk of bone marrow disorders, particularly in association with retrovirus infection.

Supportive management

Where cats are dehydrated, which is very common in hemoplasmosis, fluid deficits and electrolyte imbalances should be addressed with isotonic crystalloids. Conversely, chronically anemic cats are at risk of fluid overload due to increased circulating volume that, particularly in the presence of occult cardiac disease, may precipitate congestive heart failure. Care should be taken where history, physical examination findings and clinicopathological results do not indicate the degree of chronicity. Following re-hydration, red cell parameters should be reassessed, as the severity of anemia may have been exacerbated.

In cats where the anemia has rapidly developed or where the anemia is severe ($\leq$12%), cardiovascular compromise may be evident, despite correction of fluid deficits. In such cases, administration of either whole blood, or preferably packed red blood cells, should be considered. AB system blood-typing should be performed prior to the administration of the first unit, and a unit of the same type administered. Cross-matching has been recommended from four days after administration of the initial unit, although in some cats antibodies can be detected, by way of a positive cross-match result, as early as two days post-transfusion [49]. Due to the presence of non-AB system feline erythrocyte antigens [50] some experts have advocated cross-matching the initial blood product, in addition to all subsequent ones; however, a recent study did not find that cross-matching the initial blood product altered outcome or post-transfusion red blood cell parameters [51].

The role of corticosteroids in the management of hemoplasmosis is unclear. In *M. haemofelis*-infected cats, clinical signs have resolved without their administration [19,21], suggesting that they are not necessary. Moreover, immunosuppression induced by corticosteroid administration has been used in experimental studies with the aim of enhancing infection or inducing recrudescence of bacteremia [15,44,52]. Further studies are required to determine whether corticosteroids at any dose rate are detrimental or beneficial; however, it is generally accepted that they may be indicated pending confirmation of diagnosis.

Specific management - hemoplasmosis

Like their relatives the mucosal mycoplasmas, hemoplasmas lack a cell wall, and as such are inherently resistant to antibiotics that are cell wall active such as the β-lactams (e.g. penicillin-derivatives and cephalosporins) and glycopeptides (e.g. vancomycin). In addition, due to the parasitic nature of the hemoplasmas, they are predicted to be inherently resistant to anti-metabolite antibiotics (e.g. trimethoprim sulphonamide), as are the mucosal mycoplasmas. In contrast, both tetracyclines and fluoroquinolones have been shown to have efficacy for the treatment of clinical hemoplasmosis in cats [19,30,35-37,53-57].

Antibiotics of the tetracycline group (e.g. oxytetracycline, doxycycline) inhibit protein synthesis by blocking access of transfer RNA molecules to the 30S ribosomal subunit and are considered bacteriostatic. In contrast, antibiotics of the fluoroquinolone group (e.g. enrofloxacin, marbofloxacin, pradofloxacin) interfere with DNA replication by inhibiting the bacterial gyrase and / or the topoisomerase IV enzyme and are considered bactericidal. Experimentally, there is a well-characterized antagonism between fluoroquinolone ciprofloxacin and tetracycline in the killing of the model bacterium *Escherichia coli* [58,59]. However, one report documents unpublished studies of *Mycoplasma genitalium* that provide evidence of a synergistic effect between moxifloxacin and doxycycline in moxifloxacin-susceptible strains [60].To date, there are no data to support concurrent administration of fluoroquinolones and tetracyclines for feline hemoplasmas other than a single case report of the successful management of hemoplasmosis in a human [61].

In cats experimentally infected with *M. haemofelis*, rapid resolution of the clinical signs of hemoplasmosis occurred during the administration of both tetracycline (doxycycline 5 mg/kg orally twice daily for 14 days) and fluoroquinolones (enrofloxacin 5 mg/kg or 10 mg/kg orally once daily for 14 days; marbofloxacin 2 mg/kg orally once daily for 28 days; marbofloxacin 2.75 mg/kg orally once daily for 14 days; pradofloxacin 5 mg/kg or 10 mg/kg orally once daily for 14 days) [19,35,36,52]. Where different antibiotics, and doses, were compared, none were found to be clinically superior [35,36], potentially due to low numbers in each treatment group. Simultaneous administration of tetracyclines and fluoroquinolones was also associated with a significant and marked decrease in *M. haemofelis* load. It should be noted that these studies included untreated control cats, all of whom recovered without antibiotic administration, with some being administered fluid therapy if dehydrated, inappetent or severely anemic. Clearance of *M. haemofelis* infection was not achieved in the majority of cases treated with doxycycline, enrofloxacin or marbofloxacin. Of the cats treated with pradofloxacin, although 50% (n=6) were initially negative by PCR, subsequently repeated testing found three of these to have very low hemoplasma copy numbers present, with a further two becoming intermittently PCR positive following immunosuppression [36].

In cats experimentally infected with '*Ca.* M. haemominutum', administration of marbofloxacin (2 mg/kg orally once daily for 28-days) resulted in a significant decrease in hemoplasma load, compared to the non-treatment control group, although this response was less dramatic, delayed and was non-sustained compared to the response of *M. haemofelis*-infected cats to marbofloxacin [19,57].

One experimental study assessed the response of '*Ca.* M. turicensis' infection to antibiosis, in a limited number of cats (n=3). After doxycycline treatment (10 mg/kg once daily for 14 days), one cat showed a sustained decline in hemoplasma load following treatment. The other two cats were initially administered marbofloxacin (2 mg/kg once daily for 10 days), with negative '*Ca.* M. turicensis' PCRs obtained 4-days later; however, detectable hemoplasma loads were obtained on the 10th day of treatment. Treatment was switched to doxycycline (10 mg/kg once daily for 14 days), and negative '*Ca.* M. turicensis' PCR results were obtained 14-days later. Neither of these two cats were monitored further. A single case study describes the clearance of '*Ca.* M. turicensis' from a naturally infected cat following a course of doxycycline (10 mg/kg once daily for 14 days), with no subsequent recrudescence [34].

The European Advisory Board on Cat Diseases (ABCD) currently recommends doxycycline (10 mg/kg orally once daily or 5 mg/kg orally twice daily) as a first-line therapy for clinical hemoplasmosis,

typically for 2–4 weeks [62]. Due to the risk of esophagitis and stricture formation [63], particularly with the hyclate form of doxycycline, tablets or capsules should be administered followed by water or a small amount of food. Fewer adverse effects are reported when doxycycline is administered in divided doses or in the monohydrate form, such as those found in some liquid or paste formulations [62]. Fluoroquinolones could be considered as an alternative, although enrofloxacin should be avoided where possible due to risks of retinal toxicity and acute blindness [64], particularly where alternative fluoroquinolones are available.

Specific management – chronic carrier status

A recent study described a treatment protocol that consistently cleared *M. haemofelis* infection in a small number (n=15) of chronically infected cats [44]. Furthermore, following immunosuppression recrudescence was not detected. The protocol comprised a course of doxycycline (5 mg/kg orally twice daily for 28 days) followed, if organisms were still detectable in the blood, by a course of marbofloxacin (2mg/kg orally once daily for 14 days). Delays of up to four weeks' duration between the administration of doxycycline and marbofloxacin were not detrimental to outcome. It should be noted that to determine clearance of infection, the *M. haemofelis* qPCR was performed in quadruplicate / triplicate to increase assay sensitivity (from ≥200 copies per mL to ≥50-66 copies per mL) on a weekly basis. However, neither drug is labelled for the treatment of hemoplasmosis in the cat and the suggested course durations for both doxycycline and marbofloxacin were relatively long. These findings should not be extrapolated to other feline hemoplasmas.

In case reports of canine and human hemoplasmosis with clinical remission and apparent clearance of infection, as demonstrated by serial PCR testing, treatments have comprised extended administration of either tetracycline alone [65] or combinations of tetracycline and fluoroquinolone [61]. A dog infected with *M. haemocanis* was administered doxycycline for nearly 3 months [65]. A second canine case infected with *M. haemocanis,* clinically responded to extended courses of antibiotics (2 months oxytetracycline followed by 8 months enrofloxacin), although persistent clearance of infection was not achieved [66]. Both canine cases involved dogs that had been splenectomized for non-neoplastic disease. In a human infected with '*Candidatus* Mycoplasma haemohominis' there was a marked clinical improvement (resolution of hemolytic anemia) and achievement of non-detectable hemoplasma levels in blood in response to doxycycline alone; however, relapse occurred following discontinuation [61]. Clinical cure followed 6-months' worth of both doxycycline and moxifloxacin, with a 1-year follow-up.

Group considerations

Arthropods, particularly the cat flea *Ctenocephalides felis*, have been implicated in the transmission of feline hemoplasmas; however, confirmation of transmission by fleas by experimental demonstration has been limited [67,68]. Currently, regular administration of anti-ectoparasitic treatment seems prudent in cats. Aggressive interactions leading to the subcutaneous inoculation of infected blood have also been implicated in transmission of hemoplasma infection [69]; therefore indoor housing and separation of cats between which aggressive interactions are known to have occurred also seems sensible. Spread of infection has also been reported between cats housed together in the absence of arthropods and without reports of aggressive interactions [68]. Therefore, where the aim is to maintain hemoplasma-free status, uninfected cats should not be housed with hemoplasma-infected cats.

Summary/Discussion

Hemoplasmosis due to *M. haemofelis* infection is an uncommon cause of moderate-severe anemia in cats. In contrast, '*Ca.* M. haemominutum' is detected in approximately one in every five cats, and can result in mild anemia during acute infection; however, as a carrier state is common, concurrent disease should be investigated in clinically anemic cats. Infection may be exacerbated by immunosuppression, therefore prompt identification of the infecting hemoplasma species and appropriate antibiotic administration (e.g. tetracycline; fluoroquinolone) is necessary. Although cats with hemoplasmosis respond rapidly to antibiosis and supportive care, initial mono-therapy courses rarely result in clearance of infection. A protocol now exists for the clearance of the most pathogenic feline hemoplasma *M. haemofelis*.

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